

What is claimed is:

1. A method for detecting a nucleic acid target sequence in a sample, comprising the steps of:

(a) forming a nucleic acid target sequence;

(b) forming a primer pair for amplifying said target sequence in a polymerase chain reaction, said primer pair including a forward primer and a reverse primer;

(c) labeling said forward primer with a first dye;

(d) labeling said reverse primer with a second dye, said first and second dyes interacting to produce a signal when they are located within a specific proximity and are subjected to an energy stimulus;

(e) introducing a quantity of said forward and reverse primers, a quantity of thermostable nucleic acid polymerase, a quantity of deoxynucleotide triphosphates, and a quantity of said nucleic acid target sequence in aqueous reaction medium, in a reaction vessel;

(f) initiating a polymerase chain reaction in said reaction vessel to amplify said target sequence, said polymerase chain reaction producing a first quantity of double stranded amplification product of said target sequence, said amplification product incorporating said

forward and reverse primers on opposite complementary strands of said amplification product and said first dye of said forward primer and said second dye of said reverse primer located within said specific proximity and producing a signal on application of said energy stimulus, detectable by a measuring device.

2. The method of claim 1, wherein said first and second dyes are fluorescent dyes.
3. The method of claim 1, wherein said first and second dyes are selected from a group that includes luminescent and phosphorescent dye moieties.
4. The method of claim 1, wherein said signal involves fluorescent resonance energy transfer.
5. The method of claim 1, wherein said specific proximity is not greater than about 100 Å°.
6. The method of claim 1, wherein said measuring device is a spectrofluorimeter.
7. The method of claim 1, wherein said target sequence has a length of up to about 130 nucleotides.
8. The method of claim 1, wherein said target sequence has a length in the range of about 25 nucleotides to about 100 nucleotides.

9. The method of claim 1, wherein said amplification product has a length in the range of no greater than about 130 base pairs.

10. The method of claim 1, wherein said first dye is a donor fluorophore and said second dye is an acceptor fluorophore, and wherein said application of said energy stimulus produces fluorescent resonance energy transfer (FRET) between said first dye and said second dye, and said signal is a fluorescent emission signal produced by said second dye following FRET.

11. The method of claim 1, wherein a control reaction is completed, using water in place of said target sequence, establishing a background signal level which may be compared to said signal produced by said amplification product.

12. The method of claim 1, comprising an additional step of conducting a melting temperature analysis of said amplification product, following said step of initiating a polymerase chain reaction.

13. The method of claim 1; wherein said target sequence includes a mutation point, and said amplification product includes a copy of said mutation point.

14. The method of claim 13, wherein said step of forming said first and second primers includes structuring the nucleotide sequences of said primers to hybridize to nucleotide sequences flanking said target sequence, and wherein said signal is analyzed to determine the length of said target sequence.

15. The method of claim 1, comprising the steps of:

(a) forming a second primer pair for amplifying a second target sequence, said second primer pair including a second forward primer and a second reverse primer;

(b) labeling said second forward primer with a third dye;

(c) labeling said second reverse primer with a fourth dye, said third and fourth dyes interacting to produce a second signal when they are located within a second specific proximity and are subjected to a second energy stimulus;

(d) at the time of said first step of introducing, second introducing a quantity of said second forward and second reverse primers and a quantity of said second target sequence in said aqueous reaction medium in said reaction vessel; and

(e) at the time of said first step of initiating, second initiating a second polymerase chain reaction to amplify said second target sequence producing a first quantity of a second double stranded amplification product of said second target sequence, said second amplification product incorporating said second forward primer and said second reverse primer on opposite complementary strands of said second amplification product, and said third dye and said fourth dye located within a specific proximity and producing a second signal on application of said second energy stimulus, detectable by said measuring device, permitting simultaneous amplification, detection and quantification of two target sequences.

16. The method of claim 1, wherein said target sequence is a sequence of messenger RNA (mRNA), and wherein said step of initiating a polymerase chain reaction includes a first cycle that includes annealing said forward primer to said mRNA target sequence and producing a

complementary strand of DNA (cDNA), and includes further cycles amplifying said cDNA strand, the double stranded amplification product incorporating said forward and reverse primers and producing a signal on application of an energy stimulus, detectable by a measuring device.

17. A method for detecting a nucleic acid target sequence in a sample, comprising the steps of:

- (a) forming a nucleic acid target sequence having a length of up to 130 nucleotides;
- (b) forming a primer pair for amplifying said target sequence in a polymerase chain reaction to produce a double stranded amplification product, said primer pair including a forward primer and a reverse primer;
- (c) preparing a dye for labeling said double stranded amplification product in solution when such amplification product is formed;
- (d) introducing a quantity of said forward and reverse primers, a quantity of thermostable nucleic acid polymerase, a quantity of deoxynucleotide triphosphates, a quantity of said dye, and a quantity of said target sequence in an aqueous reaction medium;
- (e) inducing an amplification reaction, producing a quantity of amplification product derived from said target sequence, said dye thereafter labeling said amplification product; and
- (f) detecting said labeled amplification product with a measuring device.

18. The method of claim 17, wherein said nucleic acid target sequence has a length in the range of from about 25 nucleotides to about 100 nucleotides.

19. A method for forming a primer pair, including a forward and reverse primer, for use in

nucleic acid amplification, comprising the steps of:

(a) forming a forward primer and a reverse primer, each primer including a sequence of nucleotides with a 3' end and a 5' end, and having a reactive -OH functionality on the 3' end, each said sequence of nucleotides corresponding to a portion of a nucleic acid target sequence to which said primer anneals during amplification; and

(b) labeling each of said forward primer and reverse primer with a member of a fluorescent dye pair, each member of the dye pair connected to said primer at a nucleotide located from one to seven nucleotides from the 3' end of said sequence of nucleotides, said dye pair members producing a detectable signal when said forward and reverse primers are incorporated into the opposite complementary strands of a double stranded amplification product in which said primers are located within a specific proximity.